

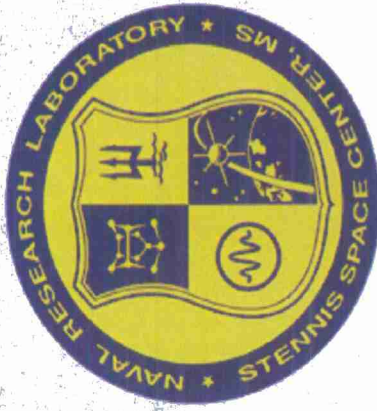
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Marine MIC of Mild Steel - Electrochemical analysis of model corrosion communities

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24-28 June 2012, ICMCF

16

Seattle, WA, USA

20121203013

Background

- FeRB and FeOB are routinely co-located in iron corrosion products
- FeRB may enhance corrosion under some circumstances or have a passivating effect on corrosion in others (Herrera & Videla, 2009; Dubiel Hsu, Chien, Mansfeld and Newman, 2002; Larsen, Little, Nealson, Ray, Stone and Tian 1998)
- FeOB have been shown to enhance corrosion in pure, marine cultures (McBeth et al, 2011)

Hypothesis:

- cultures containing both FeOB and FeRB will have enhanced corrosion in comparison with monoculture experiments or abiotic controls

Approach:

- Explore combined effects of FeOB and FeRB in pure culture experiments
- Build a model for community interactions and synergistic effects in marine corrosion communities
- Try to elucidate what microbial processes contribute to formation of tubercles on mild steel

Treatments

- Abiotic control (no added bacteria, sterile)
- Iron-oxidizing bacterium (FeOB) – strain DIS-1, a *Zetaproteobacteria*
- Iron-reducing bacteria (FeRB) – *Shewanella frigidimarina* and *Shewanella japonica* (from Biffinger Group, NRL DC)
- FeOB + FeRB – mix of strain DIS-1 and *Shewanella frigidimarina* or *Shewanella japonica*

Experimental Design

- Coupons embedded in resin
- Triplicate geochemical experiment samples, and triplicates for profilometry analyses
- Autoclaved in bottles
- 100 ml artificial saltwater medium added
- Inoculated with bacteria (FeOB, FeRB, or both, and Abiotic controls)
- Incubated at $27 \pm 2^\circ\text{C}$
- Sampled for aqueous and solid Fe(II) and Fe(total) concentrations, cell counts, pH, Eh, contamination at regular intervals

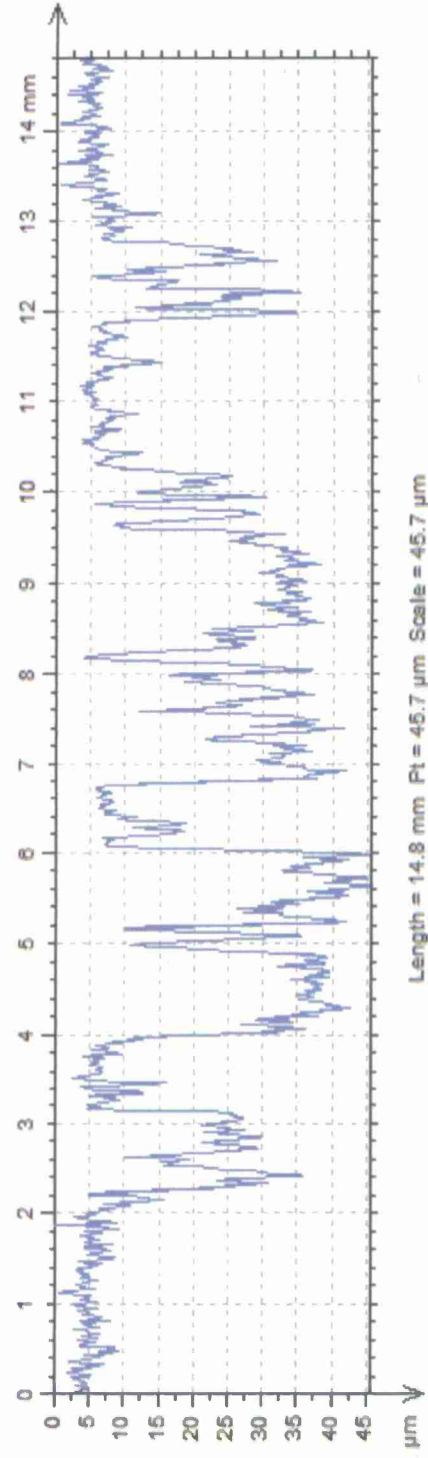
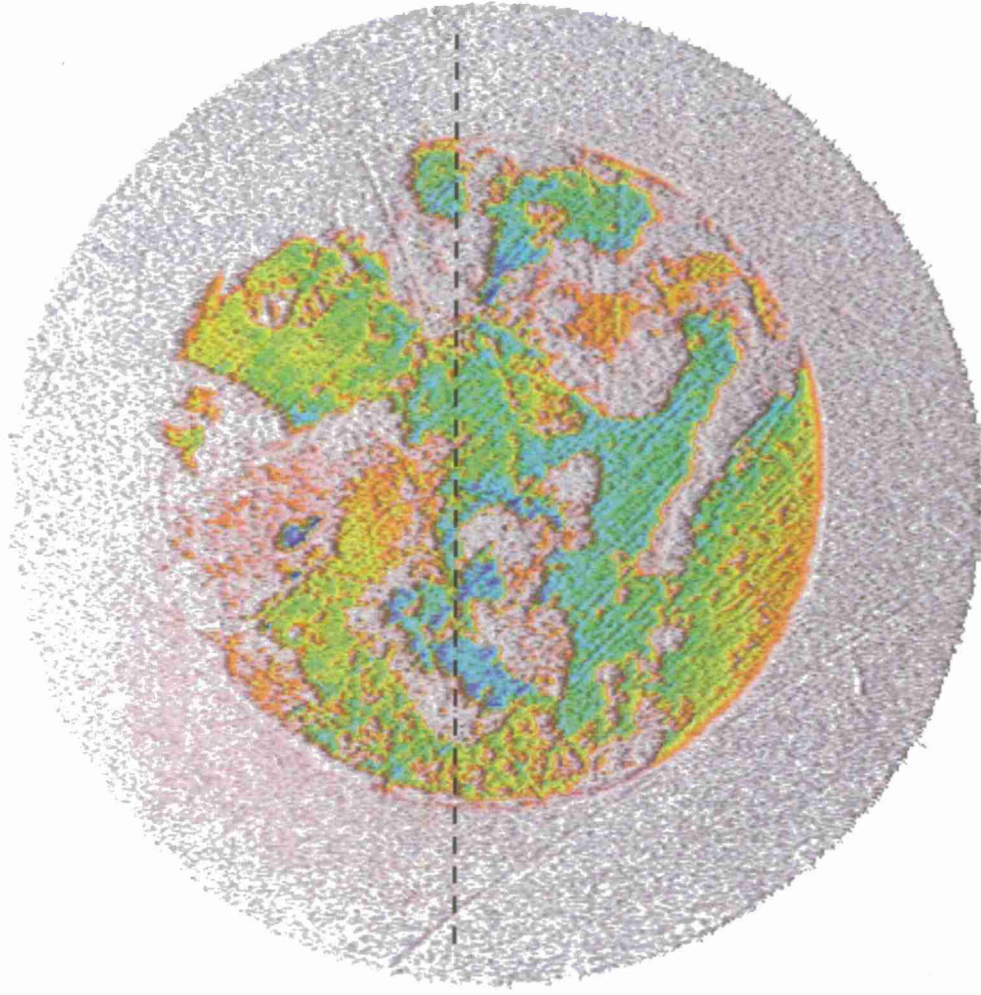
FY'11 data

Aerobic

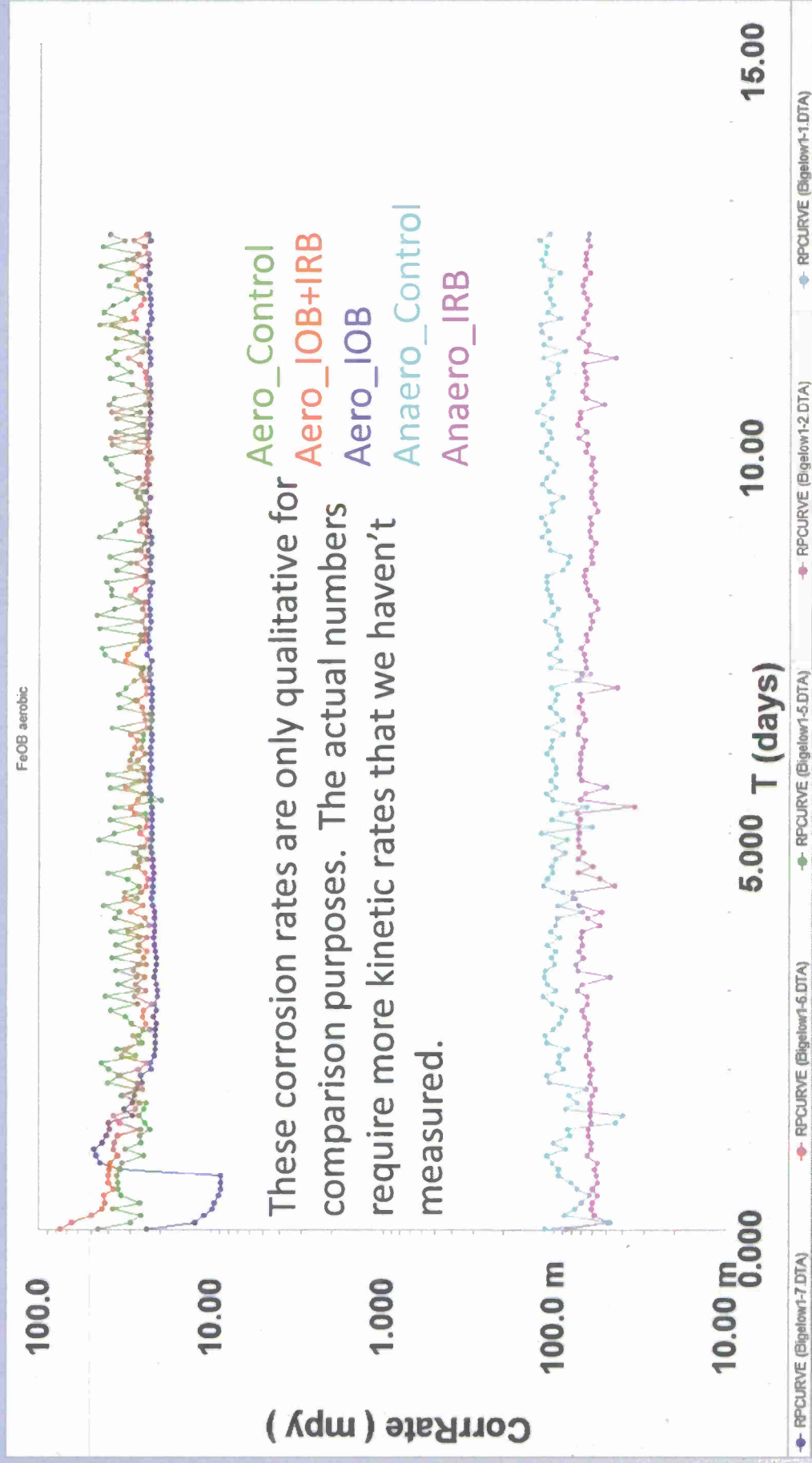
IOB+IRB

Surface (mm²)
Volume (mm³)
Max. depth/height (μm)
Mean depth/height (μm)

Hole
98.7
1.33
49.4
13.7



Corrosion Rate (mils per year)



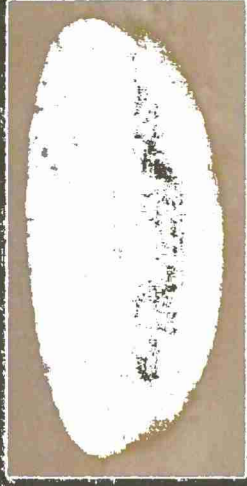
FY'12 Corrosion products at end of experiment: treatments differ, less adherent iron oxides in presence of FeRB



Abiotic



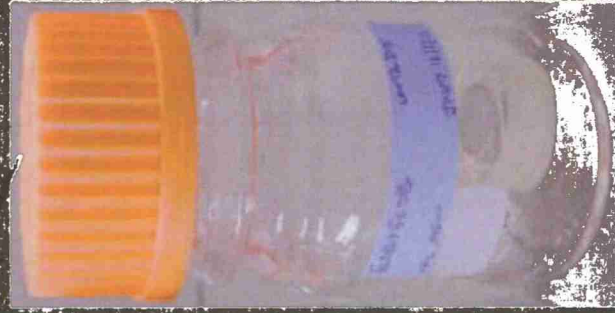
FeOB



FeRB



FeOB+FeRB

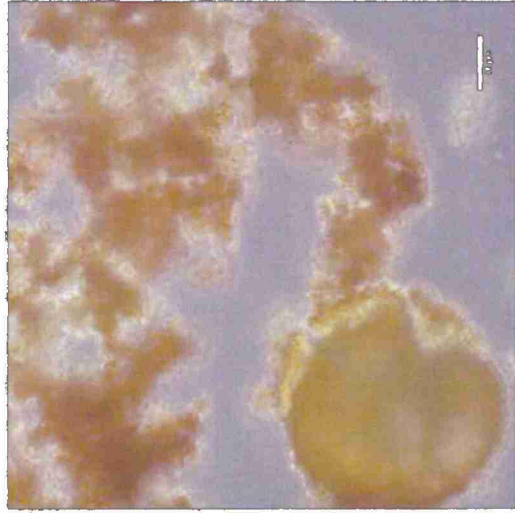


To

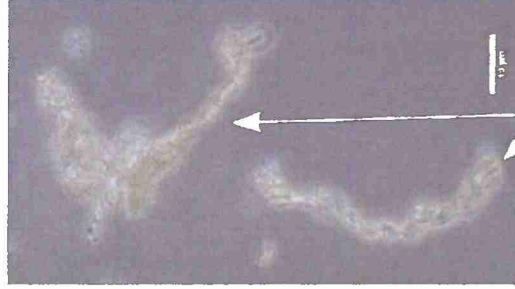


T final (13 days)

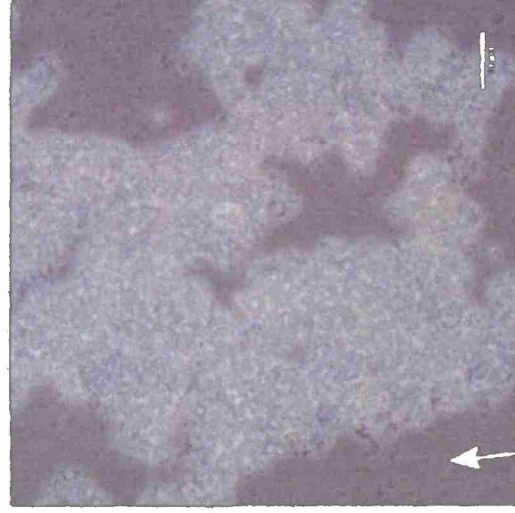
Abiotic



FeOB



FeRB



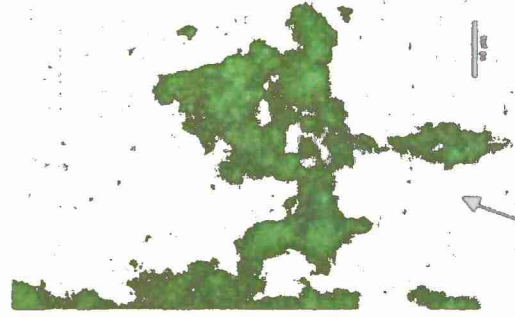
planktonic
S. frigidimarina
cells

stalks

FeOB + FeRB



stalks



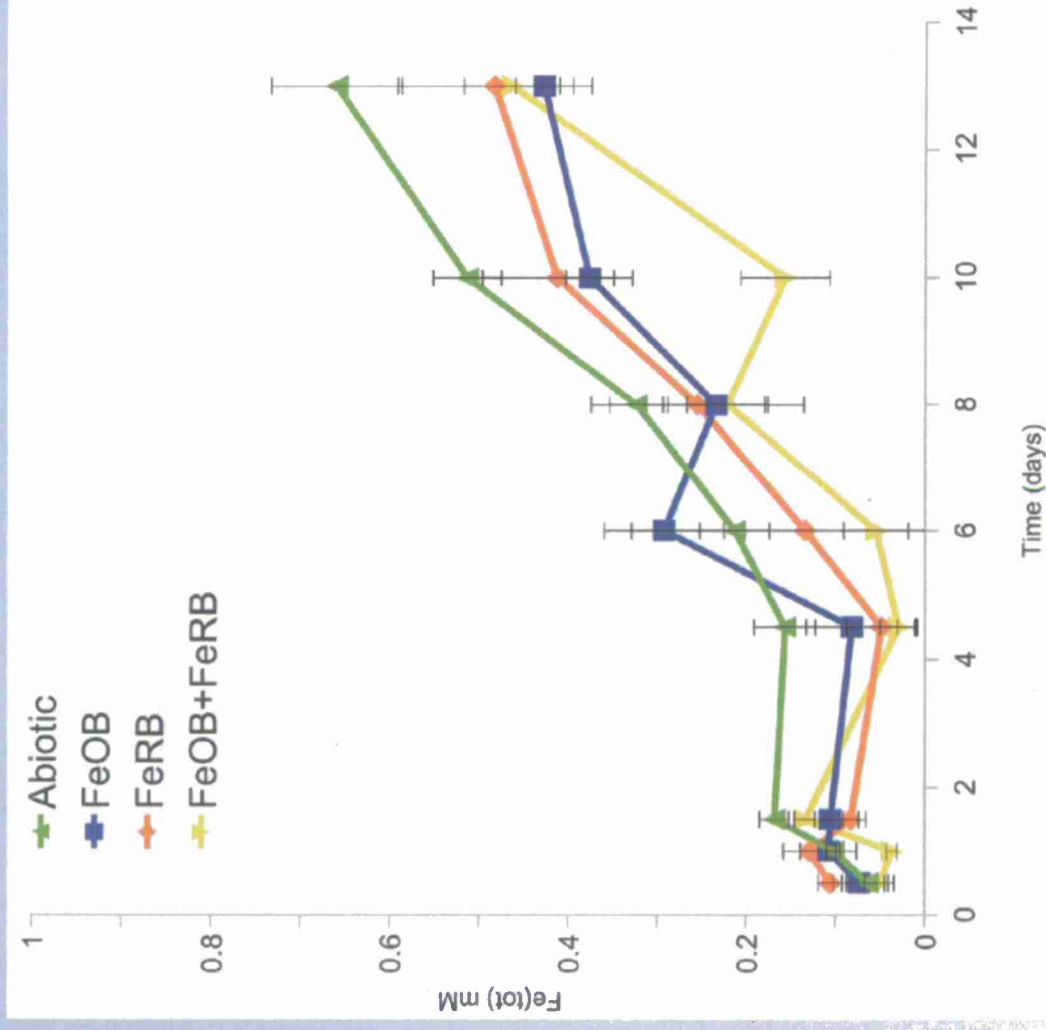
planktonic
cells

Geochemical Results

-Total iron concentration (measure of total corrosion) increasing for all samples over course of experiment

-not a significant difference between the treatments

-note adhesion of corrosion products to the Abiotic and FeOB treatments may have affected the Fe(tot) readings from those sets of samples



Notes

- Comparing these results with McBeth et al 2011 results, differences in conditions and results:
- Different FeOB strain: strain DIS-1 appears to form more adherent biofilms than strain GSB2 (used in McBeth et al 2011)
- Much larger mild steel surface area in McBeth et al 2001 experiments

Notes

- All bacteria grew, no evidence of significant contamination during experiment
- pH ca 7-8
- eH of the water: quite high throughout, decreased more in the samples containing FeRB
- Overall corrosion on coupons did occur over course of 13 day experiment, measurable increase in total iron in all samples.



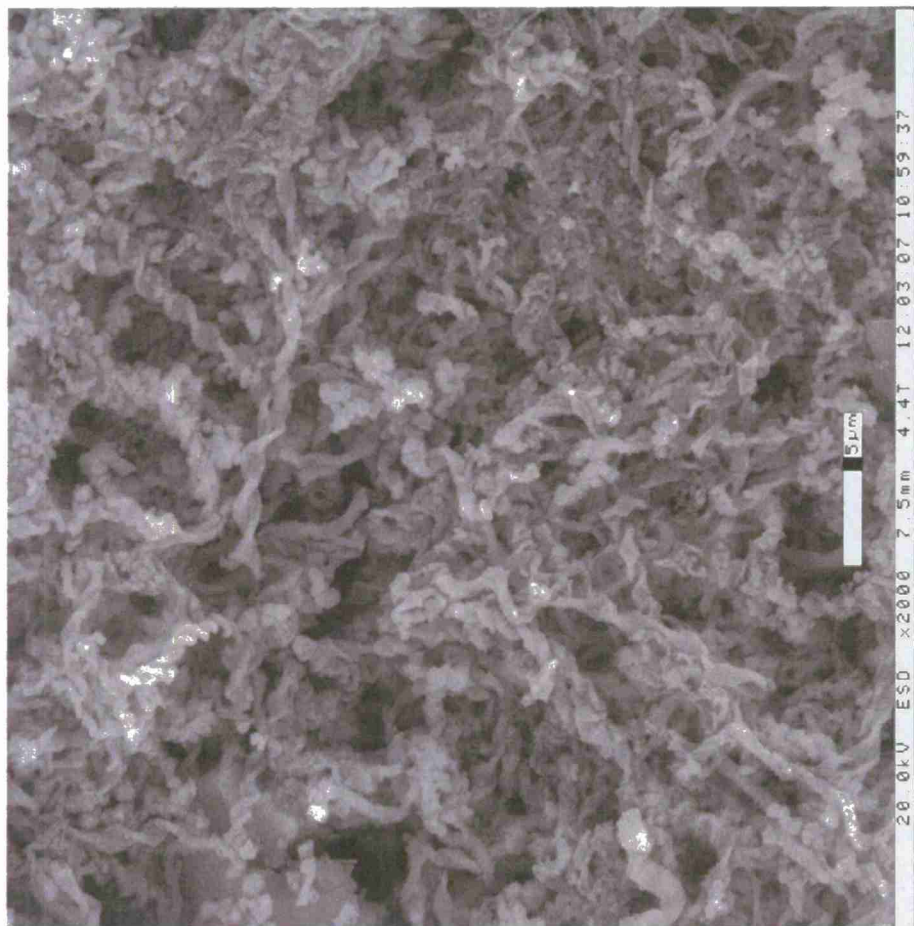
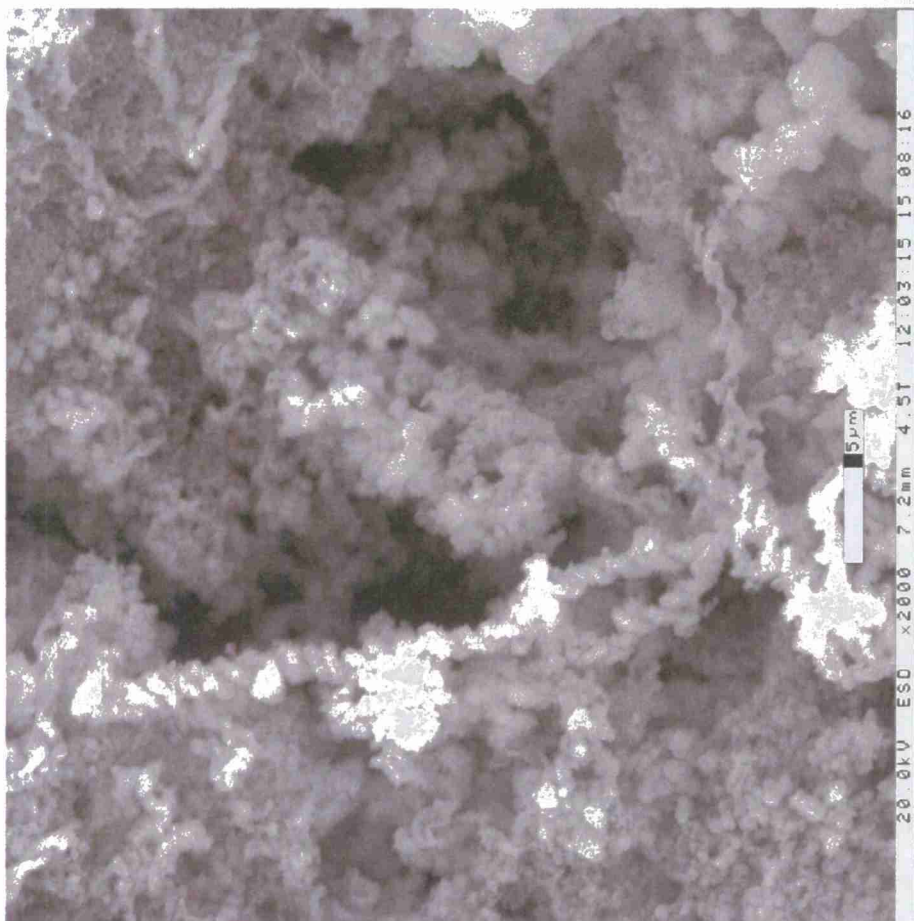
IOB

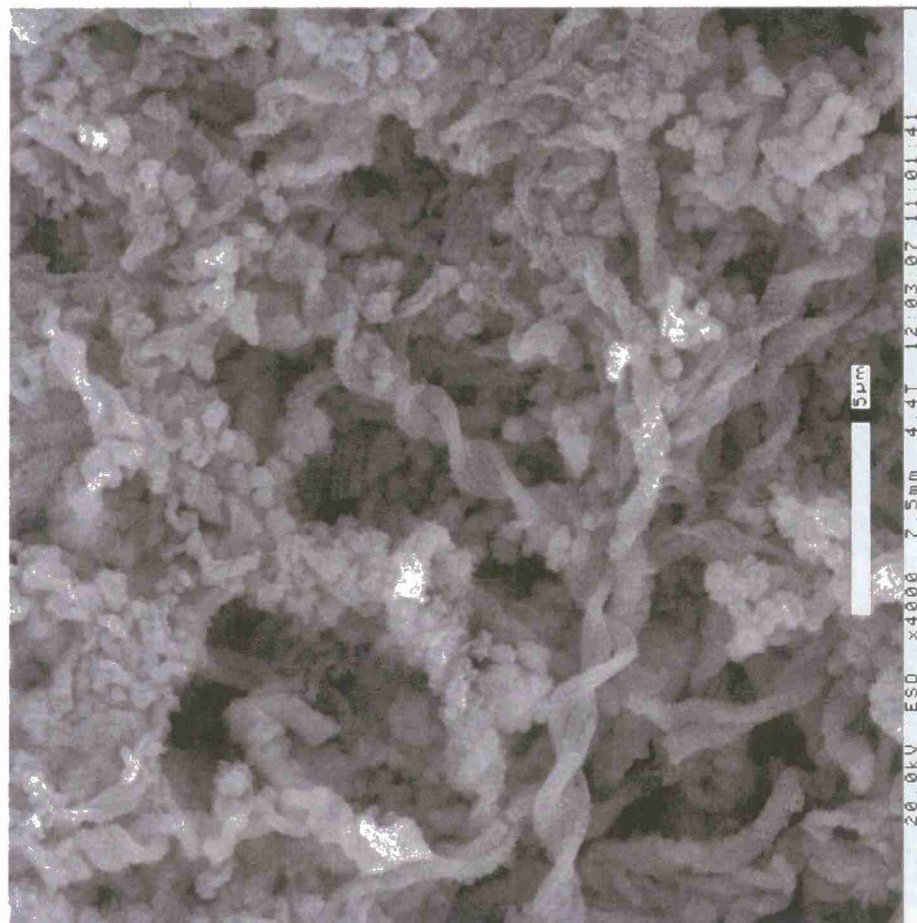
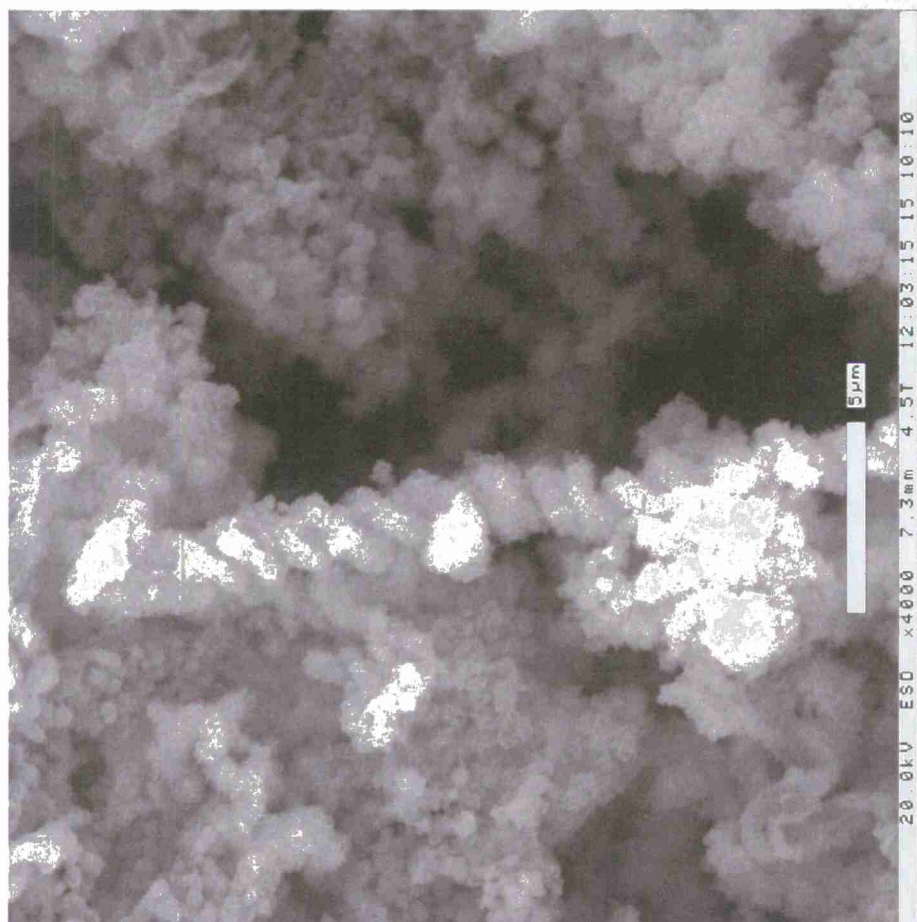


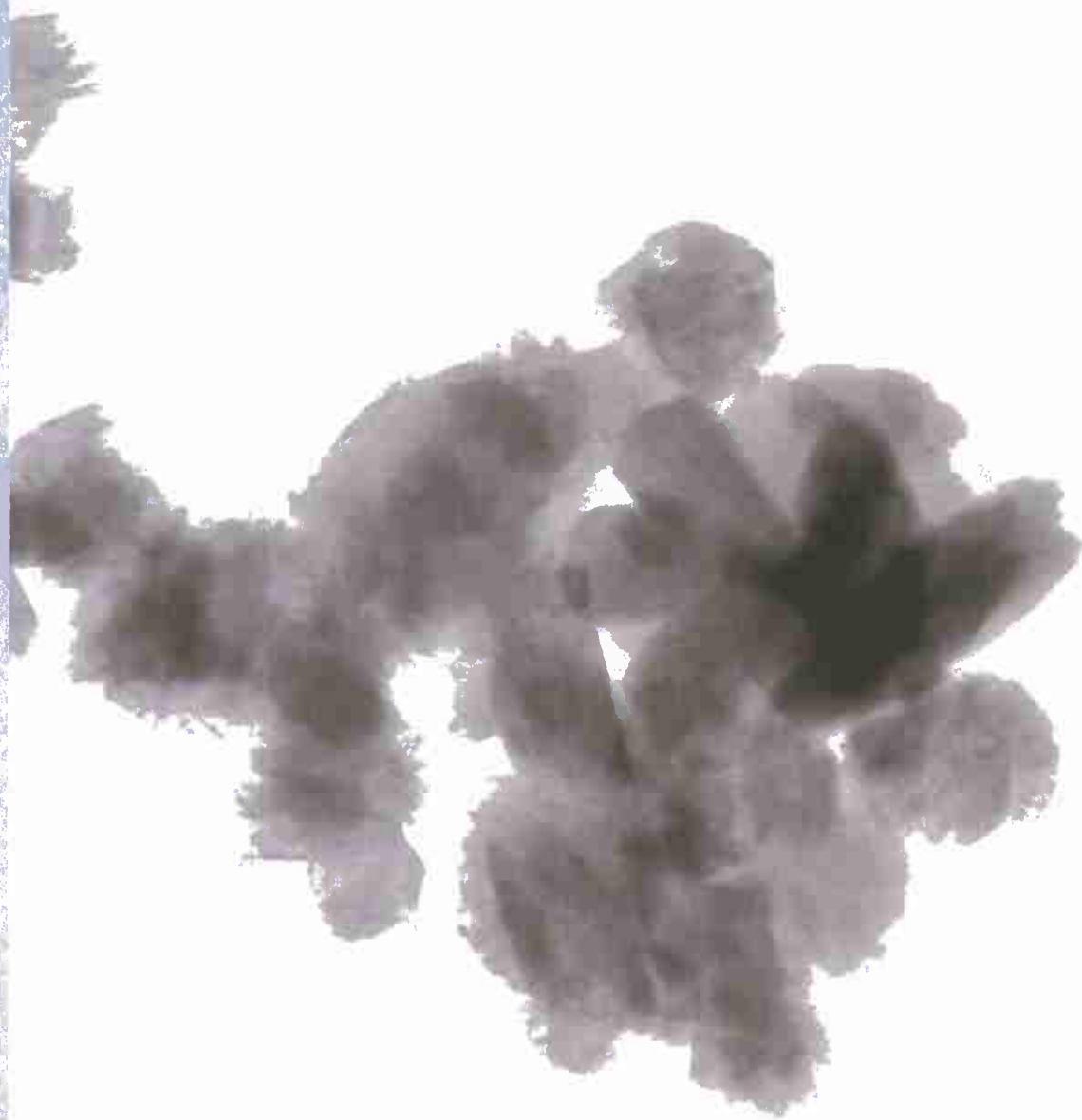
IRB



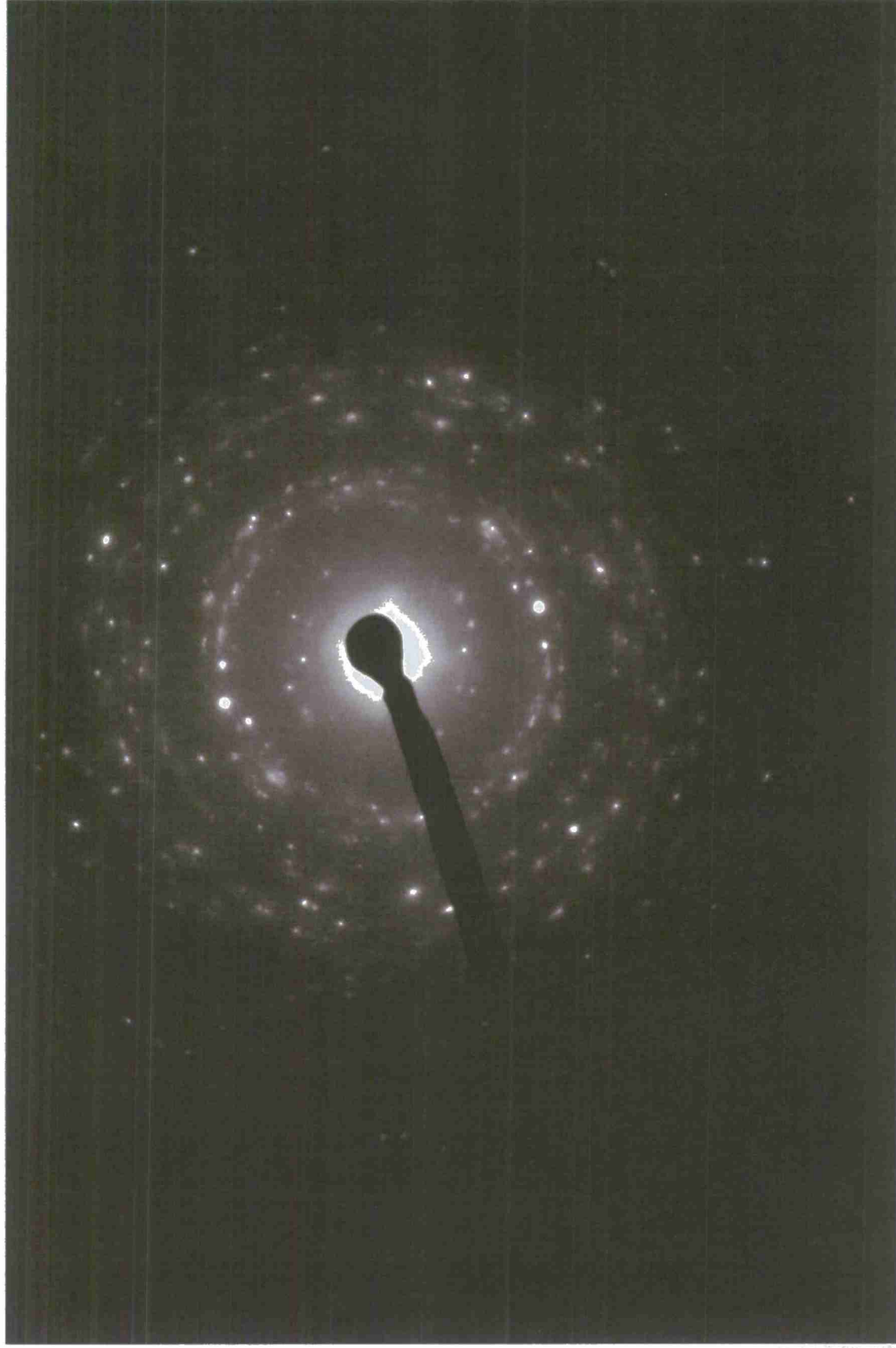
IOB + IRB



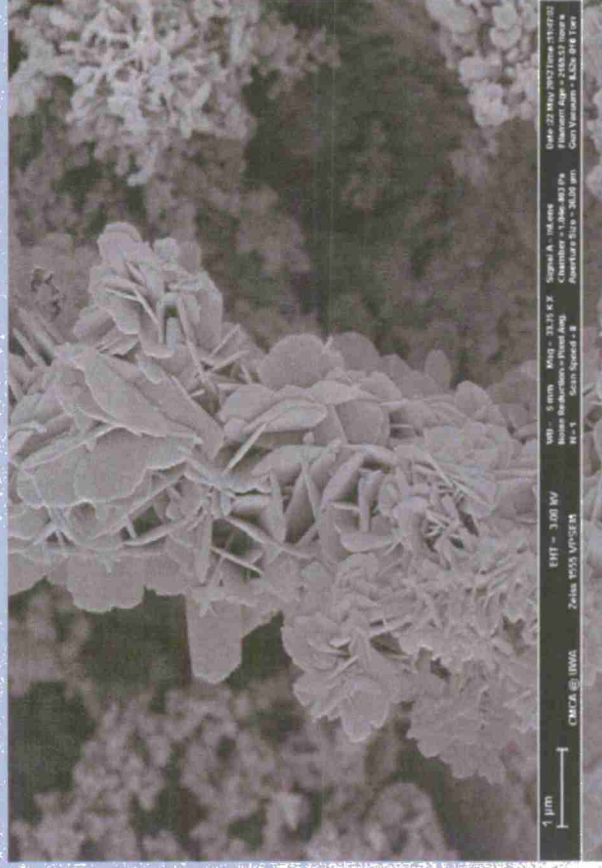




100 μm



- Selected area electron diffraction (SAED) performed by Kayley Usher (CSIRO Land and
- Water, WA, Australia) and Martin Saunders (CMCA)



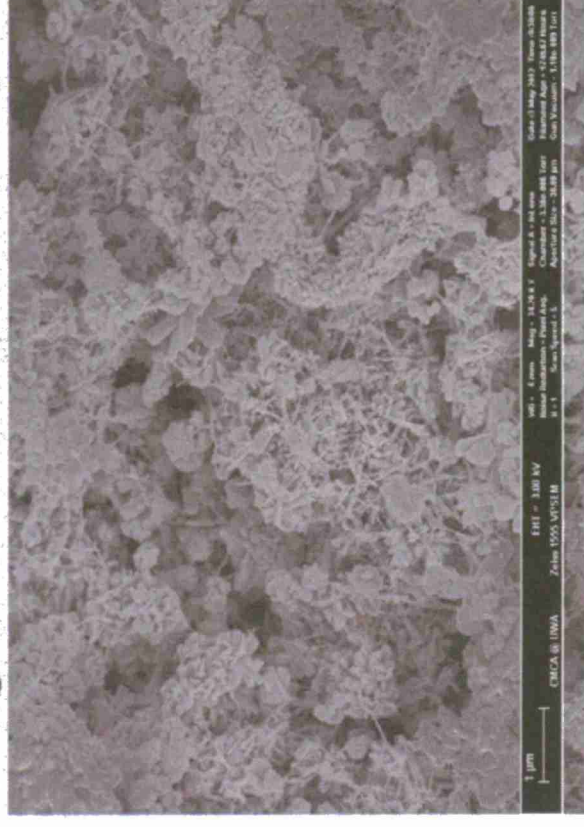
Control: hematite



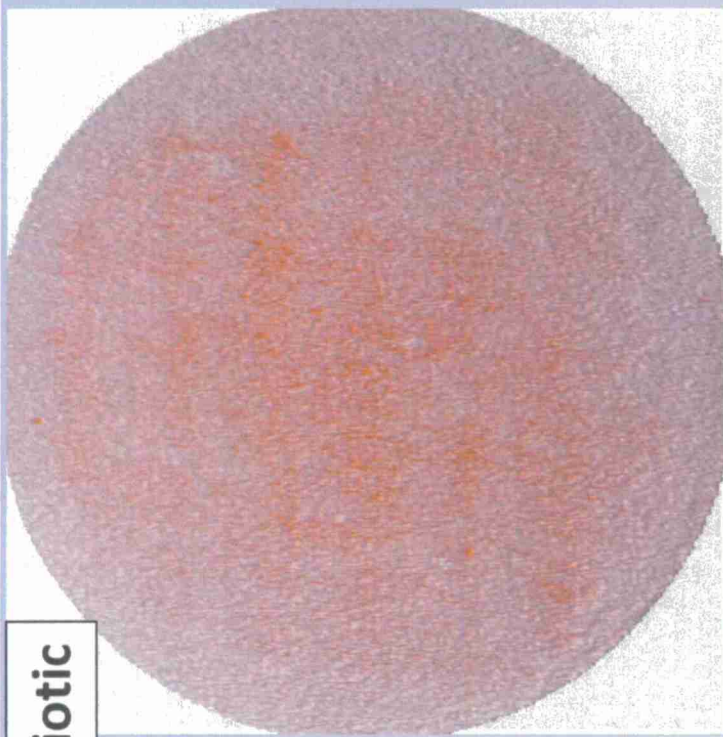
IRB: geothite, lepidocrocite, magnetite and hematite



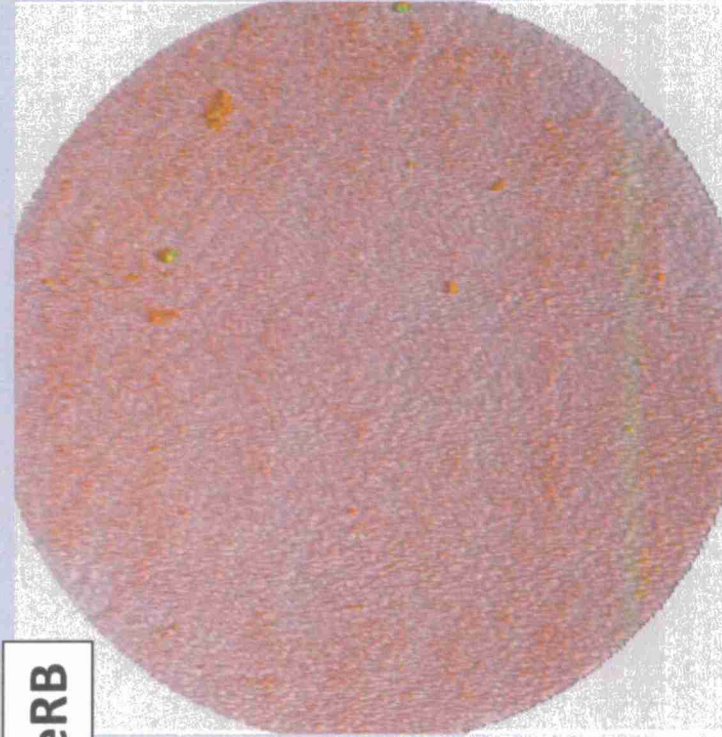
IOB: geothite only with simple morphology, and no long crystals or twinning



IOB plus IRB: geothite only with a number of different crystal morphologies including some long twinned crystals



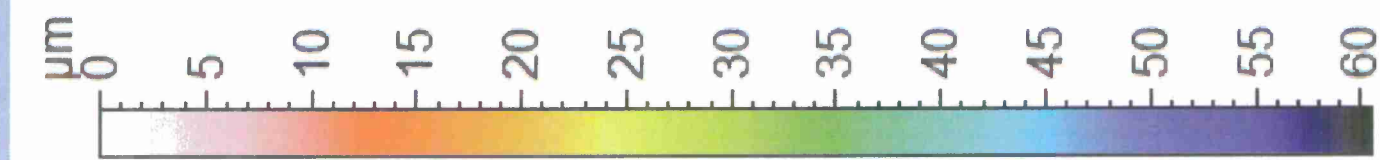
Abiotic



FeRB



FeOB+FeRB



Observations

- Under all circumstances carbon steel surfaces exposed to IRB + IOB are rougher than surfaces exposed to either alone.
- Oxides on stalks produced by IOB are removed by IRB
- Mineralogies produced by IRB on carbon steel are complex.

MIC-3

MILD STEEL CORROSION IN NEARSHORE MARINE ENVIRONMENTS - ASSESSING THE PRESENCE OF IRON-OXIDIZING BACTERIA AND CHARACTERIZING THE OVERALL BACTERIAL COMMUNITY

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Little is known about the microbial ecology of corroding steel in marine environments^{1,2} or of the natural abundance of iron-oxidizing bacteria (FeOB) in these systems. We hypothesized that coastal sediments are reservoirs for the marine FeOB 'Zetaproteobacteria' (Zetas), and that they can colonize and become numerically abundant on mild steel surfaces. A 40 day time series incubation was conducted in a salt marsh (summer 2010). Corrosion community DNA was extracted and analyzed for bacterial diversity with tagged pyrosequencing (V4 region, 16S rRNA gene). Several relevant communities were quantified using qPCR: bacteria and archaea³ and Zetas⁴ using 16S rRNA gene specific primers, and sulfate-reducing bacteria (SRB) using a *dsrA* gene specific primer⁵. The pyrosequencing data showed the presence of Zetas in sediments and throughout the incubations on the steel samples. Iron oxyhydroxide stalk biosignatures were observed on samples, further evidence that these sequences likely represent FeOB. Relatives of the H₂-oxidizing genus *Hydrogenophaga* and members of the family Rhodobacterales were also identified as important members of the biocorrosion community and were present both on steel and in sediments. Gene copies assessed with qPCR remained fairly constant in sediments during the study, and Zetas were ca 10-fold lower than SRB. Zetas colonizing the steel increased rapidly over the first 10 days, exceeding copies quantified in the sediment by an order of magnitude. The SRB numbers on the steel were 10 fold lower than in sediments during the first days of incubation, but increased to near the sediment levels by 40 days. This work illustrates that coastal sediments are a reservoir for Zetas who, though numerically low in sediments, can quickly colonize environments where free Fe(II) is abundant.

References: (1) McBeth JM *et al* (2011) *Appl Env Microbiol* 77: 1405-12; (2) Dang H *et al* (2011) *Env Micro* 13: 3059-74; (3) Takai K & Horikoshi K (2000) *Appl Env Microbiol* 66: 5066-72; (4) Kato S *et al* (2009) *Env Microbiol* 11: 2094-2111; (5) Ben-Dov E *et al* (2007) *Microb Ecol* 54: 439-51.

MIC-4

MARINE MIC OF MILD STEEL - ELECTROCHEMICAL ANALYSIS OF MODEL CORROSION COMMUNITIES

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Brenda.Little@nrlssc.navy.mil (*corresponding author)

Previous studies have shown that Fe(II)-oxidizing bacteria (FeOB) and Fe(III)-reducing bacteria (FeRB) are involved in steel corrosion, and enhance mild steel corrosion in laboratory studies^{1,2}. The objective of this work was to determine the electrochemistry of mild steel challenged with single strains of FeOB and FeRB vs co-cultures of FeOB and FeRB. Batch experiments containing mild steel coupons in marine medium were conducted in green flasks. Pure and mixed cultures of marine FeOB (*Mariprofundus ferrooxydans* strain M34)³ and FeOB (*Geothermobacter* sp. strain HR-1)⁴ were used in each system, and controls containing no added FeOB and FeRB were also prepared. Pure FeOB were grown in an aerobic bulk medium and pure FeRB were grown under anaerobic conditions. Corrosion rates were monitored electrochemically, and following incubation, steel surfaces were evaluated with ESEM and profilometry. An FeOB and FeRB co-culture was successfully grown in an bulk aerobic environment, and the FeOB-generated iron oxide stalks in this treatment appeared denuded in comparison with those formed in the pure FeOB system. Profilometry demonstrated less uniform corrosion attack in the presence of FeOB and FeRB co-culture compared to all other exposures. Electrochemically monitored polarization resistance suggested that all aerobic corrosion rates were similar and orders-of-magnitude higher than anaerobic corrosion rates. Further work developing model systems for assessing the individual and collective influences of key microbes on corrosion include incorporation of sulfate-reducing bacteria.

References: (1) McBeth JM *et al* (2011) *Appl Env Microbiol* 77: 1405-12; (2) Herrera LK & Videla HA (2009) *Int Biodet & Biodeg* 63: 891-95; (3) McAllister SM *et al* (2011) *Appl Env Microbiol* 77: 5445-5457; (4) Emerson D (2009) *Geomicro J* 26: 639-47.

